Synthesis, Crystal Structure and Biological Activity of 1-(4-(4-Ethoxybenzyl)piperazin-1-yl)-2-(2-methylphenoxy)ethanone

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ABSTRACT One novel phenoxyacetamide derivative ($C_{22}H_{28}N_2O_3$, M_r = 368.47) has been synthesized and determined by means of NMR spectroscopy, high resolution mass spectra and single-crystal X-ray diffraction. The single crystal belongs to the monoclinic system, space group *Cc* with a = 14.910(3), b = 14.592(3), c = 38.683(8) Å, β = 100.37(3)°, V = 8279(3) Å^3, Z = 16, D_c = 1.183 g/cm^3, F(000) = 3168, μ = 0.079 mm^{-1}, Mo$K\alpha$ radiation (λ = 0.71073 Å), the final R = 0.0508 and wR = 0.0666 for 4120 observed reflections with $I > 2\sigma(I)$. There are four independent molecules in an asymmetric unit cell. The four symmetry-independent molecules have a variety of different conformations indicating considerable conformational freedom. The bioassay results indicated that the title compound displayed effective activities against glutamine-induced neurotoxicity in PC12 cells and significantly prolonged the survival time of mice subjected to acute cerebral ischemia.

Keywords: phenoxyacetamide; crystal structure;synthesis; anti-ischemic activity;
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1 INTRODUCTION

Acute ischemic stroke (AIS) is a disease that is characterized by neuronal dysfunction and apoptosis induced by the interruption of blood supply resulting from the occlusion or rupture of blood vessels[1, 2]. It is the most common cause of death and a major cause of disability worldwide[3, 4]. In China, the costs associated with the treatment for ischemic stroke are a large financial burden.

The last three decades have witnessed a new surge of basic science investigations on the

pathophysiological events following AIS. Although so far clinical trials have repeatedly failed, neuroprotection is still a promising option for AIS treatment[5].

Our previous studies discovered that phenoxyacetyl diphenylmethylpiperazine analogs often show neuroprotective activity in Glu-induced PC12 cells[6]. Preliminary structure-activity relationship (SAR) study indicated that replacing diphenylmethylpiperazine with benzylpiperazine moiety probably improved the neuroprotective effect. In the present investigation, we prepared a new phenoxyacetyl benzylpiperazine derivative, namely 1-(4-(4-ethoxybenzyl)piperazin-1-yl)-2-(2-methylphenoxy)ethanone (Scheme 1), and detected its crystal structure by X-ray diffraction method. The neuroprotection of the title compound was also characterized.

2 EXPERIMENTAL

2. 1 Materials and apparatuses

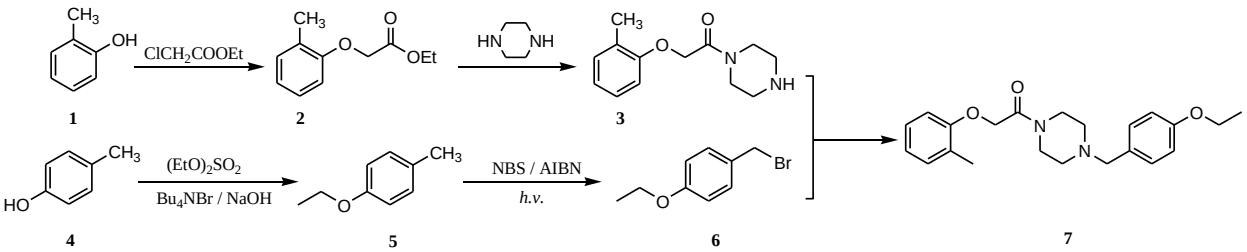
All chemicals, reagents, and solvents were of analytical grade and used without further purification. Melting point was determined on an electrothermal digital apparatus model WRR-401 (Shanghai, China) without correction. The ^{1}H and ^{13}C NMR spectra were measured on a Bruker ACF-500 Instrument (Bruker) with $CDCl_3$ as the solvent and TMS as the internal standard (chemical shifts are expressed as δ values, J in hertz). High resolution mass spectra (HRMS) were recorded on a MALDI Micro MX instrument (Waters). Fetal bovine serum (FBS) was obtained from HyClone (Logan, UT, USA). Horse serum (HS), penicillin and streptomycin were obtained from Gibco BRL (Div. of Invitrogen, Gaithers-burg, MD, USA). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Sigma Chemical Co. (St. Louis, MO, USA).

2. 2 Synthesis

The title compound was conveniently synthesized as outlined in Scheme 1. The intermediates **2** and **3** were synthesized according to our previous work[6]. The key intermediate 4-ethoxybenzyl bromide **6** was prepared by free-radical bromination of commercially available 4-ethoxytoluene **5** with little excess of *N*-bromosuccinimide (NBS)[7]. The title compound 1-(4-(4-ethoxybenzyl)piperazin-1-yl)-2-(2-methyl-phenoxy)ethanone **7** was obtained as the following description. Under a nitrogen atmosphere, 2-methylphenoxyacetyl piperazine (**3**, 17.0 mmol), 4-ethoxybenzyl bromide (**6**, 17.0 mmol), and triethylamine (2.4 mL, 17.0 mmol) in acetonitrile (90 mL) were heated to reflux for 20 h. The reaction mixture was filtered

and the solvent was removed in vacuo. The crude product was purified by column chromatography on silica gel (100～200 mesh) using a mixture of ethyl acetate and petroleum ether (V/V 2:1) as eluent to afford target compound **7** as a white solid, yield 67.8%. m.p.: 77.3～80.2 ℃. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 7.19～7.13 (m, 2H, Ar-H), 7.14～7.11 (m, 2H, Ar-H), 6.89～6.81 (m, 4H, Ar-H), 4.67 (s, 2H, OCH_2CO), 4.03 (q, J = 7 Hz, 2H, $PhOCH_2CH_3$), 3.63～3.60 (m, 4H, $CON(CH_2)_2$), 3.43 (s, 2H, $PhCH_2N$), 2.41～2.39 (m, 4H, $PhCH_2N(CH_2)_2$), 2.23 (s, 3H, $PhCH_3$), 1.41 (t, J = 7 Hz, 3H, $PhOCH_2CH_3$). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 16.23, 42.24, 45.52, 51.96, 52.62, 53.10, 60.23, 67.96, 111.03, 121.27, 126.63, 126.95, 127.19, 129.84, 129.88, 130.90, 131.14, 131.48, 138.78, 155.95, 166.56. HRMS (ESI, m/z): Calcd. for $C_{22}H_{28}N_2O_3$ $[M + H]^+$ 369.2178, found 369.2176.

Single crystals of the title compound suitable for X-ray diffraction were grown by slow evaporation of acetonitrile at room temperature.



Scheme 1. Synthesis of the title phenoxyacetamide compound 7

2. 3 X-ray crystal structure determination

A colorless single crystal of the title compound with dimensions of 0.30mm × 0.20mm × 0.10mm was selected for X-ray diffraction studies. The data were collected on an Enraf-Nonius CAD4/PC four-circle diffractometer equipped with a graphite-monochromatic Mo$K\alpha$ radiation (λ = 0.71073 Å) at 293(2) K using an $\omega/2\theta$ scan mode in the range of $1.07 \leqslant \theta \leqslant 25.37°$ ($0 \leqslant h \leqslant 17$, $0 \leqslant k \leqslant 17$, $-46 \leqslant l \leqslant 45$). The structure was solved by direct methods using SHELXS-97 and refined against F^2 by full-matrix least-squares method on the positional and anisotropic temperature parameters of non-hydrogen atoms, or equivalently corresponding to 973 crystallographic parameters, using SHELXL-97[8]. All H atoms were positioned geometrically and treated using a riding model by fixing the bond lengths at 0.93 CH atoms. A total of 8127 reflections were collected, of which 7919 were independent (R_{int} = 0.0723) and 4120 were observed with $I > 2\sigma(I)$. The final refinement gave R = 0.0508, wR = 0.0666 ($w = 1/[\sigma^2(F_o^2) + (0.0200P)^2]$, where $P = (F_o^2 + 2F_c^2)/3$), S = 1.008, $(\Delta\rho)_{max}$ = 0.140, $(\Delta\rho)_{min}$ = –0.120 e/Å^3.

2. 4 Biological activity

In order to investigate the *in vitro* neuroprotective activity, the title compound **7** was screened in Glu-induced PC12 cells[9, 10]. The cellular viability was assessed by MTT assay[11, 12].

PC12 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) FBS, 5% (v/v) HS, 100 U/mL penicillin, and 100 U/mL streptomycin at 37 ℃ in a humidified atmosphere of 5% carbon dioxide.

PC12 cells were inoculated in a 96-well microplate (10^5 cells/well in 100 μL medium) for 24 h. After washing with PBS, the PC12 cells were incubated with Glu (10 mmol/L) or Glu (10 mmol/L) with compound **7** (0.1, 1, 10 μmol/L) or Edaravone (90 μmol/L) for 24 h, respectively, at which the MTT (10 μL, 5 mg/mL) was added to each culture well. After incubation at 37 ℃ for an additional 4 h, the formazan crystals were dissolved by the addition of 150 μL dimethyl sulfoxide (DMSO), and the plates were shaken vigorously to ensure complete solubilization. Formazan absorbance was assessed at 490 nm by an ELISA plate reader[9, 10].

The *in vivo* anti-ischemic activity was tested using bilateral common carotid artery occlusion[13, 14]. Kunming mice of both sexes were randomly divided into groups (10 mice per group). The title compound was dissolved in aqueous 0.5% sodium carboxy methyl cellulose (CMCNa) solution before use and administered intraperitoneally (i.p.). Nimodipine was i.p. treated with 80 mg/kg as a positive control. The negative control group received normal saline (NS) in the same volume as other groups. All groups were given drugs twice a day for 3 days. Sixty minutes after the last administration, all mice were anesthetized by ether. All groups underwent the operation for common carotid artery and vagus nerves ligation. Then the survival time of mice was recorded.

3 RESULTS AND DISCUSSION

3. 1 Crystal structure of compound 7

The ^{1}H NMR, ^{13}C NMR, H RMS and X-ray diffraction data for the product are in good agreement with the structure of the title compound **7**, and its molecular structure and packing diagram are shown in Figs. 1 and 2, respectively. The selected bond distances, bond angles and torsion angles are listed in Table 1, and the corresponding lengths and angles of hydrogen bonds are given in Table 2.

Molecules of **7** crystallize in monoclinic *Cc* space group. There are four independent molecules in the asymmetric unit cell. For our convenience we named these molecules as *A*, *B*, *C* and *D*. The average bond lengths and bond angles of benzene rings are normal. The C=O bond distances of all four molecules,

C(14)=O(2) (1.234(5) Å), C(36)=O(5) (1.229(5) Å), C(58)=O(8) (1.209(5) Å) and C(80)=O(11) (1.241(5) Å), are found almost equal to a typical C=O double bond (1.20 Å)[15]. The distances of N(2)–C(14), N(4)–C(36), N(6)–C(58) and N(8)–C(80) are 1.347(6), 1.326(6), 1.328(6) and 1.349(6) Å, respectively. They are remarkably shorter than the typical C(sp^2)–N bond (1.426 Å), but closer to the C=N double bond (1.33 Å)[16].

As shown in Fig. 1, in the structure of the title compound, the piperazine rings of all four molecules are in a chair conformation with the N-$COCH_2CO$- and N-benzyl groups in the equatorial positions. The C(sp^2)–N bonds lie in the planes of the carbonyl groups of all four molecules (N(2)–C(14)–C(15)–O(3) –175.8(4) °, N(4)–C(36)–C(37)–O(6) –171.4(4) °, N(6)–C(58)–C(69)–O(9) –179.3(5) ° and N(8)–C(80)–C(81)–O(12) 178.9(4) °). The molecule contains two essentially planar phenyl rings which are not coplanar. In molecule *A*, the 4-ethoxyphenyl ring makes a dihedral angle of 57.7(3) ° with the 2-methylphenyl ring. In molecule *B*, the respective angle is 59.5(3) °, while values of 64.6(3) ° and 63.6(4) ° are observed in molecules *C* and *D*. The bond angles of the amide groups of molecules *A*, *B*, *C* and *D* are 122.7(5) °, 122.8(5) °, 123.3(5) ° and 122.0(5) °, respectively. The torsion angles of O(2)–C(14)–C(15)–O(3), O(5)–C(36)–C(37)–O(6), O(8)–C(58)–C(59)–O(9) and O(11)–C(80)–C(81)–O(12) are equal to 5.7(7) °, 6.2(7) °, –0.8(7) ° and 1.4(7) °, respectively.

Molecules of **7** show intra- as well as intermolecular hydrogen bonding. Among four molecules of **7**, similar ends of pair of molecules *A*, *C* and *B*, *D* form dimers *via* C–H ··O interactions. Piperazine hydrogen (H(11A), H(33A), H(56A) and H(78B)) and phenoxyacetyl group hydrogen (H(15A), H(37A), H(59A) and H(81B)) are involved in C–H ··O interaction. In the crystal, C–H ··O hydrogen bonds link the molecules into two crystallographically independent chains, and each chain is formed by two alternating independent molecules. These C–H ··O hydrogen bonds and weak C–H ··π interactions strengthen the integration of the three-dimensional network. These interactions are estimated to play a role in stabilizing the crystal structure.

Table 1. Selected Bond Lengths (Å) and Bond Angles (°)/Torsion Angles (°) of Compound 7

Bond	Dist.	Bond	Dist.	Bond	Dist.
O(2)–C(14)	1.234(5)	N(2)–C(14)	1.347(6)	O(3)–C(15)	1.405(5)
O(5)–C(36)	1.229(5)	N(4)–C(36)	1.326(6)	O(6)–C(37)	1.427(6)
O(8)–C(58)	1.209(5)	N(6)–C(58)	1.328(6)	O(9)–C(59)	1.404(6)
O(11)–C(80)	1.241(5)	N(8)–C(80)	1.349(6)	O(12)–C(81)	1.449(6)
Angle	( )	Angle	( )	Angle	( )
O(2)–C(14)–N(2)	122.7(5)	O(2)–C(14)–C(15)–O(3)	5.7(7)	N(2)–C(14)–C(15)–O(3)	–175.8(4)
O(5)–C(36)–N(4)	122.8(5)	O(5)–C(36)–C(37)–O(6)	6.2(7)	N(4)–C(36)–C(37)–O(6)	–171.4(8)
O(8)–C(58)–N(6)	123.3(5)	O(8)–C(58)–C(59)–O(9)	–0.8(7)	N(6)–C(58)–C(59)–O(9)	–179.3(5)
O(11)–C(80)–N(8)	122.0(5)	O(11)–C(80)–C(81)–O(12)	1.4(7)	N(8)–C(80)–C(81)–O(12)	178.9(4)

Table 2. Hydrogen Bond Lengths (Å) and Bond Angles ( )

D–H ··A	D–H	H ··A	D ··A	D–H ··A
C(11)–H(11A) ··O(8)^{i}	0.97	2.55	3.341(7)	139
C(12)–H(12B) ··O(2)	0.97	2.34	2.753(7)	105
C(15)–H(15A) ··O(8)^{i}	0.97	2.43	3.351(6)	158
C(22)–H(22A) ··O(3)	0.96	2.30	2.740(8)	107
C(33)–H(33A) ··O(11)	0.97	2.48	3.292(7)	141
C(34)–H(34B) ··O(5)	0.97	2.36	2.772(8)	105
C(37)–H(37A) ··O(11)	0.97	2.38	3.319(6)	162
C(44)–H(44A) ··O(6)	0.96	2.23	2.725(8)	111
C(55)–H(55B) ··O(8)	0.97	2.35	2.744(8)	104
C(56)–H(56A) ··O(2)^{ii}	0.97	2.55	3.365(7)	142
C(59)–H(59A) ··O(2)^{ii}	0.97	2.36	3.253(7)	153
C(66)–H(66A) ··O(9)	0.96	2.27	2.734(7)	109
C(77)–H(77A) ··O(11)	0.97	2.34	2.760(7)	106
C(78)–H(78B) ··O(5)^{iii}	0.97	2.50	3.327(7)	144
C(81)–H(81B) ··O(5)^{iii}	0.97	2.37	3.231(6)	148
C(88)–H(88A) ··O(12)	0.96	2.31	2.760(7)	108

Symmetry codes: (i) –1/2+*x*, 3/2–*y*, 1/2+*z*; (ii) *x*, 2–*y*, –1/2+*z*; (iii) 1/2+*x*,–1/2+*y*, *z*

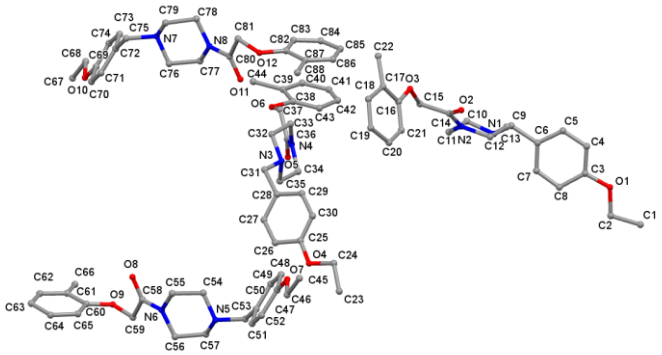


Fig. 1. Molecular structure of compound 7

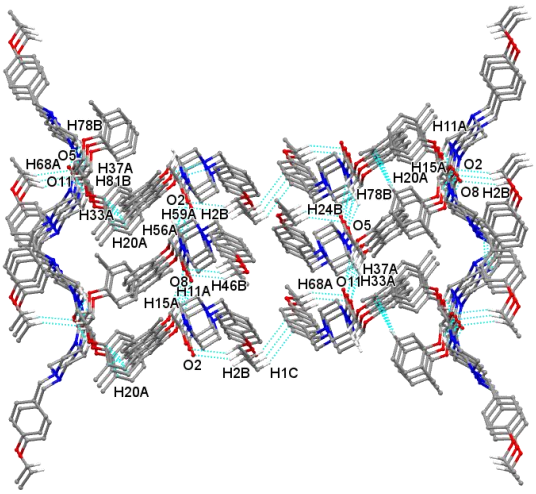


Fig. 2. Packing diagram of compound 7

3. 2 Biological activity

The *in vitro* and *in vivo* biological activities of the title compound were evaluated. The *in vitro* bioassay results showed that compound **7** can effectively protect PC12 cells against Glu-induced neuronal injury. From Table 3, it was found that cell protection at three test concentrations (0.1, 1.0, 10 μM) was 35.28%, 30.65%, and 28.93%, respectively. Remarkably, compound **7** showed good neuroprotective activity for all three test concentrations (protection > 20%). Compound **7** was observed to have the highest protection at the lowest concentrations of 0.1 μM and exhibited better neuroprotection than Edaravone at the concentration of 0.1 μM. In addition, compared with Nimodipine, the title compound **7** significantly prolonged the survival time of mice subjected to acute cerebral ischemia and decrease the mortality rate at all doses (50～400 mg/kg) tested *in vivo* (Table 4). Compound **7** exhibits effective neuroprotection and could be a lead compound for further discovery of neuroprotective agents for treating cerebral ischemic stroke.

Table 3. Neuroprotective Effects of Compound 7 against Glu-induced Neurotoxicity in PC12 Cells

Compound	Protection (%)		
	10 μmol/L	1 μmol/L	0.1 μmol/L
The title compound	28.93*	30.65*	35.28*
Edaravone (90 μmol/L)		33.0*	

*$p < 0.05$ *vs.* glutamate-treated group

Table 4. Effects of the Title Compound 7 on Survival Time of Mice Subjected to Bilateral Common Carotid Artery Ligation

Compound	Survival time (min)			
	400 mg/kg	200 mg/kg	100 mg/kg	50 mg/kg
Compound **7**	8.48 ±1.15*	7.23 ±1.09*	7.16 ±1.26*	4.36 ±0.77*
NS	2.04 ±0.61			
Nimodipine	3.52 ±1.04*			

* $p < 0.05$ *vs*. NS.

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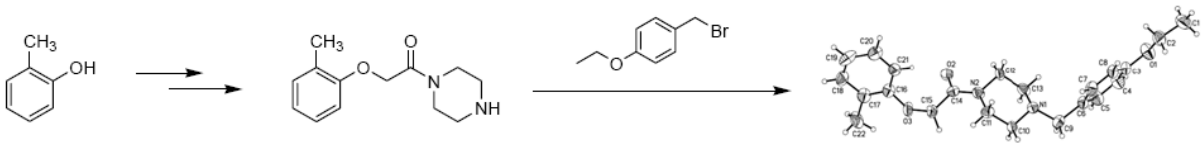

The synthesis, crystal structure and neuroprotective activity of a new phenoxyacetyl benzylpiperazine derivative are reported.